Check List the journal of biodiversity data

NOTES ON GEOGRAPHIC DISTRIBUTION

Check List 14 (2): 341–345
https://doi.org/10.15560/14.2.341

PENSOFT

First record of the Malaysian Bridle Snake, *Dryocalamus subannulatus* (Duméril, Bibron & Duméril, 1854), in Myanmar (Reptilia, Serpentes, Colubridae)

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Abstract
Dryocalamus subannulatus is reported for the first time from Myanmar. A single individual was found on the Thayawthadangyi Island Group in the Myeik Archipelago, Tanintharyi Region. Morphological features and a maximum likelihood analysis of the 16S mitochondrial gene confirm its identity. This specimen represents the first record of *D. subannulatus* north of the Isthmus of Kra.

Keywords
Distribution extension; Myanmar; Thailand; snake; *Dryocalamus*; phylogeny; morphology.

Academic editor: Ross MacCulloch | Received 15 December 2017 | Accepted 5 February 2018 | Published 16 March 2018

Citation: Lee JL, Miller AH, Connette GM, Oo KS, Zug GR, Mulcahy DG (2018) First record of the Malaysian Bridle Snake, *Dryocalamus subannulatus* (Duméril, Bibron & Duméril, 1854), in Myanmar (Reptilia, Serpentes, Colubridae). Check List 14 (2): 341–345. https://doi.org/10.15560/14.2.341

Introduction

Bridle Snakes (*Dryocalamus* Günther, 1858) are a group of 6 semi-arboreal snake species ranging across tropical Asia from India through Indochina and the Greater Sundas to the Philippines (Uetz et al. 2018). Presently only *Dryocalamus gracilis* (Günther, 1864) and *Dryocalamus davisonii* (Blanford, 1878) are known in Myanmar; both from the northern and central part of the country (Dowling & Jenner, 1988).

Dryocalamus subannulatus Duméril, Bibron & Duméril, 1854 was described as *Odontomus subannulatus* from "Padang, West Sumatra" and subsequently has been reported from other Sunda islands (Borneo, Mentawai and Riau Archipelagos), Philippines (Palawan Island), Peninsular Malaysia, Singapore, and southern Thailand (Cox, 1991, Pauwels et al. 2006). This species has 2 color morphs: a dark cross-banded one and a lighter yellow-striped one. The latter was described as the subspecies *D. subannulatus tungsongensis* by Nutphand (1986), but it was later synonymized by Pauwels et al. (2006). No records exist for this species in Myanmar. A recent (May 2017) herpetological survey in the Myeik Archipelago found a *Dryocalamus subannulatus* on Linn Lune Kyun (Kyun = Island, in Burmese), of the Thayawthadangyi

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Island Group. This specimen is the first confirmation of *Dryocalamus subannulatus* from southern Myanmar and the first record of this species north of the Isthmus of Kra. We confirm the identity of the specimen by molecular and morphological examination.

Methods

During an expedition to the Myeik Archipelago, Tanintharyi Region, a subadult female *Dryocalamus* (USNM 581990) was collected from Thayawthadangyi, Linn Lune Kyun, Tanintharyi Region, Myanmar, which we identify as *D. subannulatus* in this paper. Although additional islands of the Thayawthadangyi Island group were surveyed (Grants Island and Daung Kyun), no other specimens of this species were detected. The specimen was collected on 10 May 2017 at 20.15 hr. The collection and export of specimens was permitted by a memorandum of understanding between the Forest Department of the Ministry of Environmental Conservation and Forestry of the Republic of the Union of Myanmar and Fauna & Flora International. The specimen was later euthanized following Smithsonian Institution's National Museum of Natural History (NMNH) Animal Care and Use Committee (ACUC) protocols (2017-05). A tissue sample from the liver was placed in a DMSO/EDTA buffer solution and later frozen at –80 °C. The specimen was then injected with 10% formalin before being transferred to a 70% ethanol solution. Both the specimen and the tissue sample were deposited in the Smithsonian Institution, NMNH, Washington DC, USA (USNM).

Genomic DNA was extracted from a tissue sample and run on an Auto-Genprep 965 (2011 AutoGen, Inc.), using standard manufacturer phenol protocols. DNA was eluted in 100 µl of AutoGen R9 re-suspension buffer. We amplified the mitochondrial gene 16S rRNA subunit using the primers 16Sar (5′ CGCCTGTTTATCAAAAACAT)-16Sbr (5′ CCGGTCTGAACTCAGATCACGT, Palumbi et al. 1991) in a 10 µl reaction and at 54 °C annealing temperature for an approximately 550 bp fragment. Cycle-sequence reactions were performed in both directions, using the 16Sar-br primers using BigDye Terminator v3.1 Cycle Sequencing Kit's in 0.25 × 10 µl reactions run on and ABI3730 Sequencer (2011 Life Technologies) using the 950 chemistry. Raw trace files were edited in Geneious 9.1.5 (Kearse et al. 2012), complementary strands were aligned and edited. The sequence is deposited in GenBank under accession number MG661260. We used GenBank sequences of species *Gonyosoma oxycephalum* (Boie, 1827), KX694646; *Lycodon aulicus* (Linnaeus, 1758) KX277255; and *Lycodon fasciatus* (EU999215; Anderson, 1879) as outgroups due to their closer relationship to *Dryocalamus* (Figueroa et al. 2016). Additional species sequences of *Dryocalamus* were acquired from GenBank (*D. nympha* (Daudin, 1803), KC347360; *D. davisonii* (Blanford, 1878), KX660200, KX660228, KX660246; *D. tristrigatus* (Gunther, 1858), KX660201; *D. subannulatus* (Duméril, Bibron, & Duméril, 1854), KX660229, KX660230). We aligned the sequences using the MUSCLE (default option) in Geneious and performed maximum-likelihood (ML) analyses on the mtDNA using RAxML (v8.2.9, Stamatakis, 2014) with the rapid bootstrap inferences (1000 replicates) and subsequent GTRCAT thorough ML search.

We compared the morphology of our specimen to literature descriptions of *Dryocalamus* (Boulenger, 1893, Smith, 1943, Cox, 1991, Das, 2010). Body measurements (snout vent length, SVL; tail length, TL) were taken using a flexible ruler. Head measurements (head length, HL, defined as the length from the posterior end of the jawbone to the tip of the rostral scale; and head width, HW) and head scale measurements were taken using dial calipers to the nearest 0.1 mm. Dorsal scale row counts were made at one head length behind the head, midbody and one head length before the cloaca. Ventral scales were counted according to Dowling (1951). The tail tip was not considered for calculating the total number of subcaudals.

Results

New record. Myanmar, Tanintharyi Division, Myeik Archipelago, northeast of Done Pale Aw Village, Linn Lune Kyun, Thayawthadangyi (12°22′20.53″ N, 098°05′ 11.80″ E; WGS84). One subadult female collected by Daniel G. Mulcahy, Grant Connette, and Khin Swe Oo (USNM 581990; Fig. 1) on 10 May 2017 at 20.15 hr.

Identification. USNM 581990 is identified as *Dryocalamus subannulatus* based on the following characters: dorsal scales smooth with 15 rows midbody, single precloacal scale, temporals 2+2, loreal present and in contact with the eye and a color pattern consisting of a gray dorsum with large black body blotches on the anterior third of the body.

Description: Female; body cylindrical, slender and elongate; head round, distinct from neck. SVL 373 mm; TL 131 mm, 25.9% of entire total length; Head Length 504 mm; HL 13.6 mm; HW 7.5 mm; eye round, horizontal distance 2.2 mm, longer vertically than horizontally with round pupil; anterior end of the eye to snout 3.2 mm; parietals 1.5x frontal, longer than wide; frontal shield shaped, 2.0x prefrontals, longer than wide; prefrontals small, subrectangular, longer than wide; internasals rectangular, approx. 1.3× larger than prefrontals, wider than long. Rostral visible from above, wider than long; mental wider than long. supralabials 7/7, 3rd and 4th contacting eye; infralabials 8/8, 1st to 4th contacting upper chin shields; upper chin shields longer and wider than lower chin shields; postoculars 2/2, preoculars 1/1, loreal scale longer than wide, in contact with the eye; temporals 2+2/2+2; nasal scale divided. Dorsal scales smooth in 15–15–15 rows; ventrals 240, smooth; subcaudals 111, paired; precloacal scale single.

Head cream; eyes entirely black; underside of head plain white. Brown spots present on both temporal scales and the frontals/parietals connecting to neck; preocu-

Figure 1. Specimen of *Dryocalamus subannulatus* USNM 581990, recorded from Myanmar. Photograph by Daniel G. Mulcahy.

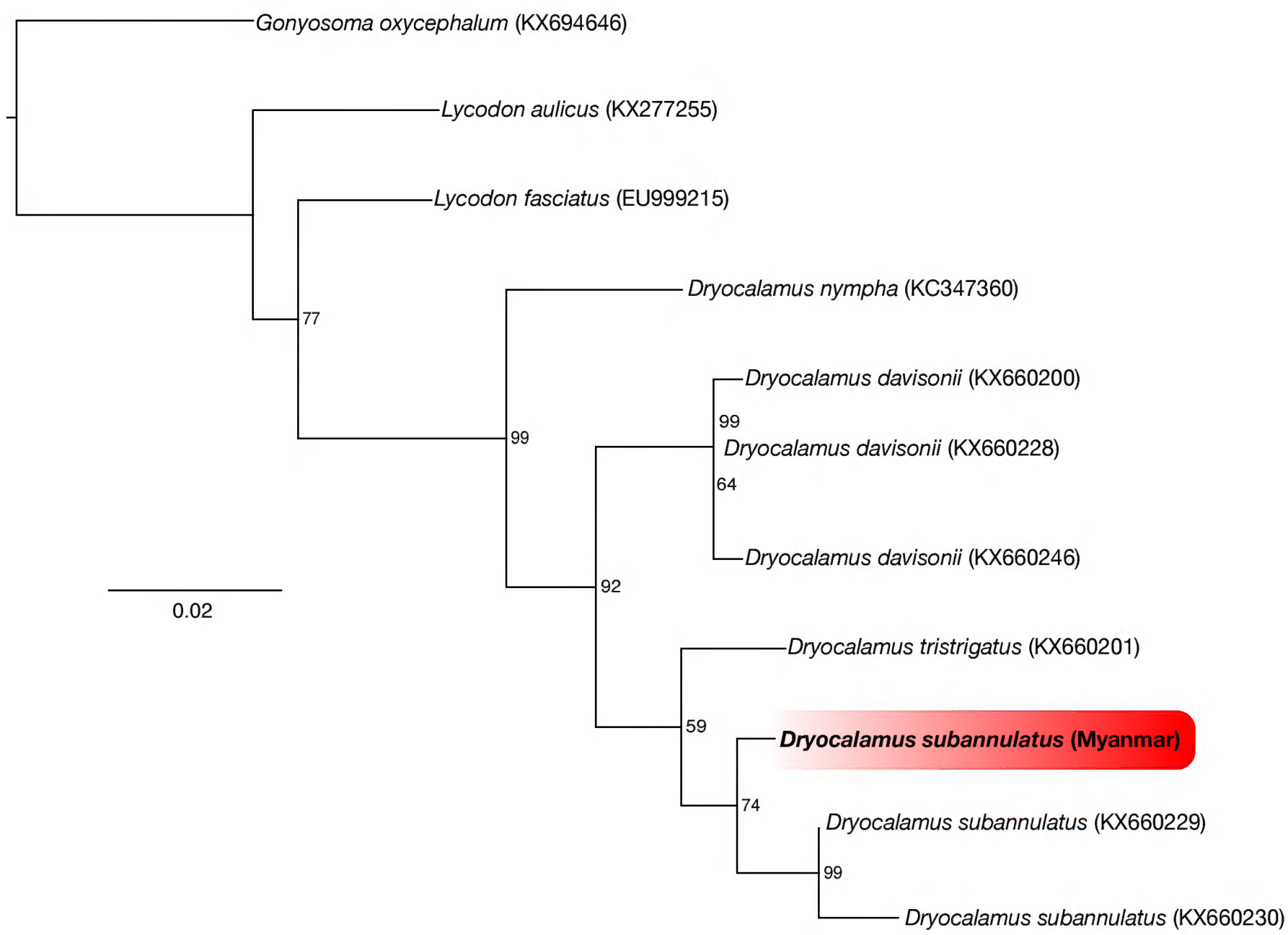

Figure 2. Maximum likelihood tree of *Dryocalamus* based on the 16S gene showing USNM 581990 specimen (highlighted in red) placed sister to *D. subannulatus* specimens. Bootstrap values are shown next to relevant nodes.

lars, nasal and rostral also brown. Anterior third of body pale grey with large black dorsal blotches. Mid-dorsal blotches divided into smaller paired dorsolateral blotches around midbody, with a series of irregular grey patches between them. A dashed lateral black line present on the posterior two-third of body, on dorsal scale rows 3 and 4. Blotches diminishing in size posteriorly. Venter of body white, edges of ventral scales have small faint dark spots; tail venter similar to the dorsum. Coloration similar in preservative.

We obtained a 530 bp fragment of the 16S mtDNA locus from USNM 581990. The ML analysis (Fig. 2) placed the Myanmar specimen sister to the other *D. subannulatus* specimens with moderate support (bootstrap value 74%).

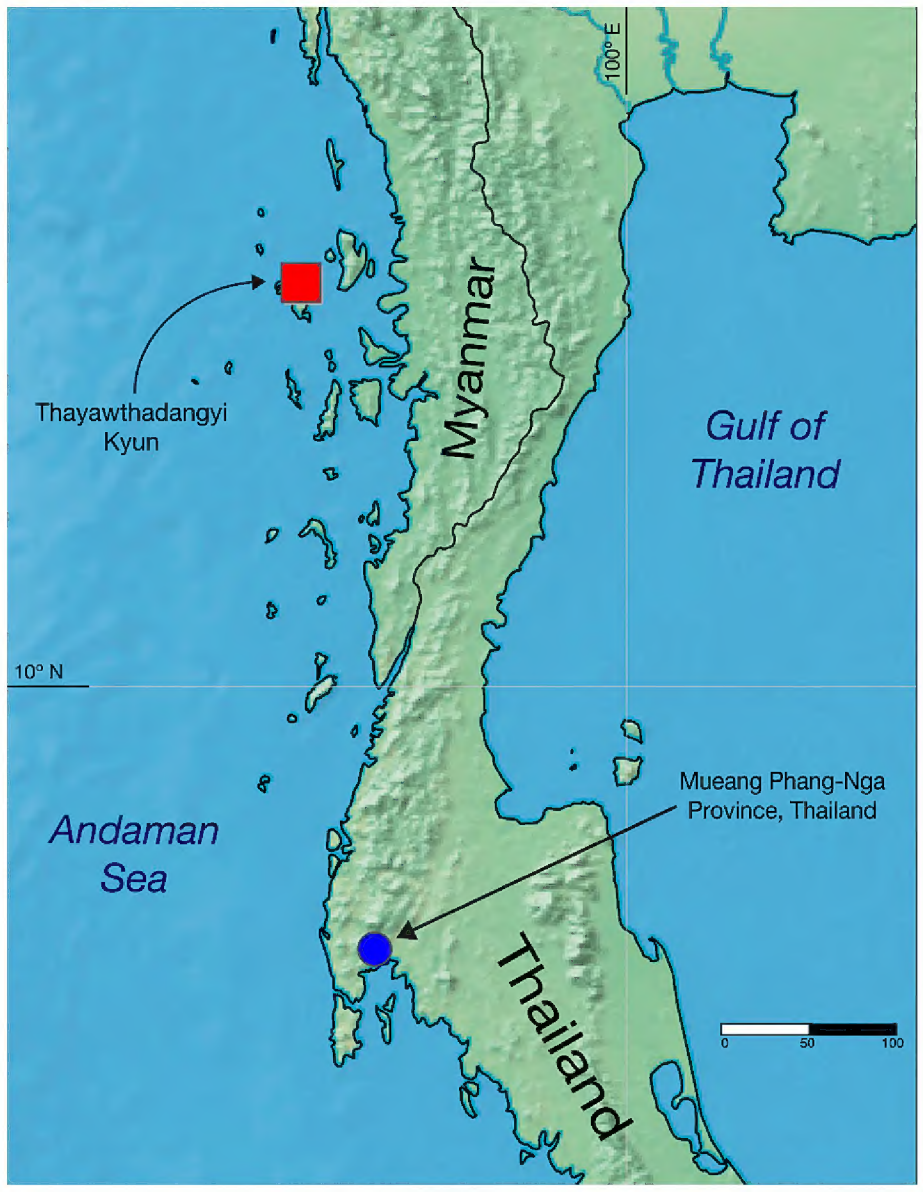

Figure 3. Distribution map showing approximate location where the new record of *Dryocalamus subannulatus* was located (red square) and the previous northernmost record in Phang Nga Province, Thailand (blue circle). Scale bar units are in kilometers. Map created by simplemappr.net.

The 3 *D. davisonii* are supported with short branches (99% and 64% respectively) and are separated from the clade containing USNM 581990 with strong support (92%). *Dryocalamus tristrigatus* (KX660201) is placed sister to all *D. subannulatus* with lower support (59%). The larger (1.9–3.9% un-corrected) genetic distance between the 2 specimens of *D. subannulatus* (KX660229-30) and USNM 581990 may be due to the geographic distance between the 2 populations, as KX660229-30 were collected approximately 750 km southeast in Peninsular Malaysia.

USNM 581990 was collected at night on the outer edge of a tree branch near a small stream. The habitat around this region is secondary lowland rainforest. No food or reproductive items were detected.

Discussion

Molecular and morphological examination confirms the identity of USNM 581990 as *Dryocalamus subannulatus*. The discovery of *D. subannulatus* in Myanmar extends the distribution of this species north of the Isthmus of Kra and approximately 430 km northeast from the nearest record (Fig. 3) in Mueang Phang Nga District, Phang Nga Province, Thailand (Pauwels et al. 2000). Furthermore, this species adds to the ever-growing number of new country records for reptiles and amphibians in Myanmar, especially in the Tanintharyi region (Wogan et al. 2008, Lee et al. 2015, Mulcahy et al. unpublished data). Interestingly, many snakes discovered in Myanmar exclusively from the Tanintharyi region like *Boiga dendrophila* (Boie, 1827), *B. drapiezii* (Boie, 1827), *Bungarus flaviceps* (Reinhardt, 1843), *Calliophis maculiceps* (Günther, 1858), *Ptyas carinata*, (Günther, 1858), *Rhabdophis chrysargos* (Schlegel, 1837), *Xenochrophis trianguligerus* (Boie, 1827) reach their northern latitudinal terminus here or in adjacent Vietnam and Cambodia (Grismer et al. 2008, Nguyen et al. 2009, Stuart et al. 2010, Lee et al. 2015). This is concordant with the biogeographic hypothesis suggested by Pauwels et al. (2003), who placed the boundary between Indochinese and Sundaland snake fauna at approximately 15° N latitude. This latitude is well north of the Isthmus of Kra and corresponds to regions 3–4 described by Inger (1999), which includes Cambodia, Southern Vietnam and Central Thailand. *Dryocalamus subannulatus* is an exception to this phenomenon since it has not been found north of the Tanintharyi Region. However, we note that there are several snake species recorded in the Tanintharyi Region which are also absent from Vietnam and Cambodia. Some of these species represent country records and shall be described in detail elsewhere (Mulcahy et al. unpublished data). Meanwhile, the diversity of reptiles and amphibians from the Tanintharyi region supports the protection of the area's evergreen rainforest and insular habitats, which currently are being destroyed at an alarming rate (Connette et al. 2017). Swift action to preserve the Tanintharyi region's unique habitats is needed to conserve and protect species from local extinction.

Acknowledgements

The herpetofauna survey is part of Flora & Fauna International's (FFI) and the Smithsonian Consortium for Research in Myanmar initiative to encourage the establishment of national parks in the Tanintharyi and to institute conservation management of this area. We thank FFI and its in-country representatives, Frank Momberg and Mark Grindley, for the sponsorship of the survey and for handling logistics, including permits. We thank Myeik University and their students, particularly Chuu Hay Mann, Zin Man Win, and Kyaw Htike Thu, and Katie Connette and Kyaw Zay Ya for participating in the field surveys. We appreciate the support of the Myanmar Forest Department of the Ministry of Natural Resources and Environmental Conservation for their administrative and personnel support and U Tin Maung Latt for escort on this survey. We are grateful to Saw Lal Htoo for accommodations and assistance on Thayawthadangyi Kyun for the survey. The Smithsonian Consortium for Research in Myanmar, particularly Melissa Songer, provided international travel and logistic support. JLL thanks the University of Maryland College of Computer, Mathematical and Natural Sciences as well as the College Park Scholars Life Sciences program for financial and logistical support.

Authors' Contributions

JLL collected morphological data, identified the species, and wrote the majority of the manuscript; DGM, GRC, and KSO collected specimens; DGM and AHM analyzed molecular data; AHM, GRZ, JLL, and DGM revised and corrected the manuscript.

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